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A Committee Report on

MYCOPLASMA GALLISEPTICUM INFECTION

IN POULTRY

commonly known as
Chronic Respiratory Disease and
Air Sac Disease in Chickens
and as Infectious Sinusitis and
Airsacculitis in Turkeys

ARS 22-81



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Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE

FOREWORD

This is the official report of a work conference on certain poultry diseases held May 28 through May 30, 1962, at the Ambassador Hotel, Washington, D. C. It embodies the conclusions and recommendations of 60 or so poultry-disease experts, disease regulatory authorities, and other scientists who attended this conference at the call of the Animal Disease and Parasite Research Division of the Agricultural Research Service.

These scientists came from all parts of the United States. Their willingness to attend and participate in the conference attest to their mutual concern about a growing problem--the apparent increase in chronic respiratory diseases of poultry and the losses suffered by the poultry industry because of these diseases.

The scientists, following guidelines laid down by the Animal Disease and Parasite Research Division, concentrated their discussions on the diseases of poultry caused by the micro-organism Mycoplasma gallisepticum. They were nonetheless able, however, to define other disease conditions sometimes associated with or confused with M. gallisepticum infection. These and other definitions, needed for a clear understanding of the discussions, appear in a glossary prepared by the scientists and included in this report.

The discussions represent the thinking of some of the best-known authorities on M. gallisepticum, and encompass the organism's characteristics and peculiarities, its detection and transmission, how it affects chickens and turkeys, and other facts about it. The discussions further reflect the scientists' experiences and successes in establishing and maintaining flocks free of M. gallisepticum infection.

To promote further the control of M. gallisepticum infection throughout the United States, the scientists also adopted certain resolutions that the Animal Inspection and Quarantine Division and the Animal Disease Eradication Division of ARS are asked to consider.

The scientists further underscored the need for more research to improve industry's chances of combating the infection successfully.

This report conveys the gist of the discussions to the poultry industry and to others who are interested in maintaining a healthy, thriving poultry industry.

The scientists, who participated in the discussions, are hereinafter referred to as the Committee. Their names appear at the end of this report.

The information in this report has been reviewed by the Animal Disease and Parasite Research Division, the Animal Disease Eradication Division, and the Animal Inspection and Quarantine Division of the Agricultural Research Service, and by the Federal Extension Service.

A Committee Report on MYCOPLASMA GALLISEPTICUM INFECTION IN POULTRY

GIVEN: The fact that the \$3.3 billion poultry industry in the United States suffers significant losses each year from certain poultry diseases caused by the micro-organism, Mycoplasma gallisepticum (see the glossary, p.13). While these losses do not threaten the existence of the industry, they are nonetheless the cause of severe economic drains to the producer and in turn increase the cost of poultry and poultry products to the consumer.

NEEDED: Research facts about these diseases that will enable the industry to cope with them successfully. Some of these facts might reflect a general statement about M. gallisepticum infection, major aspects of the infection, and how it can be controlled or eradicated.

PROGRESS: Research has progressed to the point where definite recommendations can be made to the poultry industry. This research began in June 1952, when the Animal Disease and Parasite Research Division of ARS initiated a national, cooperative research program with seven State Experiment stations. Four additional experiment stations were included in the program in 1954. Other States engaged in broiler production initiated their own research programs. This work is continuing and has brought forth much valuable information about M. gallisepticum infection. A complete bibliography covering the research is now available.

SUGGESTION: Some means should be found to bring together the scientists who conducted this research because they are the best qualified to discuss the subject. These scientists should formulate recommendations and guidelines that industry can use to reduce the incidence of the diseases and assure the public a continuing supply of wholesome poultry products.

SOLUTION: The Animal Disease and Parasite Research Division of the Agricultural Research Service brought these poultry-disease experts and others together on May 28 through May 30, 1962. During the course of the meeting, recommendations were formulated that the poultry industry should be able to use to reduce the incidence of the diseases. These recommendations form the essentials of this report.

UNDERSTANDING: The participating scientists, hereinafter referred to as the Committee, agreed to concentrate their discussion on those poultry diseases caused by the S6 type of Mycoplasma gallisepticum. Therefore, when M. gallisepticum is mentioned in this report, it is understood to be the S6 type. The glossary defines the common names for this infection. The Committee further agreed that more research is needed because many questions about M. gallisepticum infection have yet to be answered.

MAJOR ASPECTS OF THE INFECTION

Mycoplasma gallisepticum infection in poultry causes, or is associated with, the diseases and conditions defined in the glossary. It is undoubtedly the poultry industry's foremost problem. Losses directly or indirectly attributable to it certainly run as high as \$125 million a year.

The Committee unanimously pointed out, however, that these losses can be reduced if poultry producers, especially breeders, keep their flocks free of M. gallisepticum infection. The best way to do this is to establish small flocks free of the infection and build from these. The Committee further holds that these objectives are attainable and practical, if certain selection, testing, and management procedures are conscientiously and continually applied.

The Organism

The M. gallisepticum organism, as defined in the glossary, is smaller than the common bacteria, but larger than a virus. It requires more nutrients for growth than common bacteria. Because it has an extremely thin cell wall, if any, it is easily destroyed outside its host by physical and chemical agents used for routine disinfection.

The Organism and Drugs and Antibiotics

Avian Mycoplasma vary in their susceptibility to drugs and antibiotics. All known Mycoplasma are resistant to penicillin, thallium acetate, and most sulfonamide drugs. Many antibiotics (for example, streptomycin, tetracycline, erythromycin, and tylosin) inhibit Mycoplasma in test tubes. These antibiotics are also effective in live birds if extremely large doses are administered. The use of antibiotics, therefore, to control M. gallisepticum infection in very large market or breeder flocks is not economical and is not recommended. They should be used, however, when small flocks are being established free of M. gallisepticum infection.

Transmission

Research furnishes clear evidence that the transmission of M. gallisepticum from the hen through the egg to the chick or poult is the principal manner in which the disease is spread. The rate of transmission is greatest during acute infection and decreases with time.

Contact transmission within a flock is recognized to be second in importance. Experimental infection under controlled conditions demonstrate that birds will show signs from one to several weeks after becoming infected depending on (1) virulence of the strain, (2) the dose, and (3) route of exposure. Spread of the disease within a naturally infected flock may vary from days to months. Transmission of the disease may also occur within a flock without apparent clinical signs of the disease and may be accelerated by other infections.

Recorded evidence of incubator transmission of M. gallisepticum is scant. There is little doubt, however, that this method of spread should be given serious consideration.

Where birds from various sources and unknown histories and of different ages are housed in the same hatchery or on the same farm, excellent conditions exist for outbreaks of M. gallisepticum infection.

Contact with contaminated equipment or personnel is recognized as a possible means of spread. Drinking water, crates, chick boxes, footwear, and other mechanical carriers may be possible sources of spread from infected to clean flocks.

The possibility of transmitting M. gallisepticum through live poultry vaccines has not been established, but this potential hazard should be considered (see pages 4 and 8).

M. gallisepticum has been recovered from various wild fowl and its transmission from turkeys to pheasants has been recorded. The role of various wild birds in transmitting the infection to domestic poultry has not been fully established, but wild birds are not suspected as being important carriers.

Immunity

Although vaccination as a disease-control measure is widely accepted and is effective against several viral diseases of poultry, few vaccines against infections caused by bacteria and bacteria-like organisms, such as M. gallisepticum, have any value. There is therefore no effective procedure currently available to immunize poultry against M. gallisepticum infection.

Host Range of Mycoplasma

Many species of animals harbor Mycoplasma. Avian species include chickens, turkeys, ducks, pheasants, pigeons, guinea fowl, peafowl, parakeets, sparrows, quail, and partridges. Nine different serotypes of avian Mycoplasma have been reported, but the information on the host range of the pathogenic M. gallisepticum other than in chickens and turkeys is limited. While Mycoplasma have been isolated from air sac lesions in many avian species other than chickens and turkeys, their pathogenicity in domestic poultry has not been determined. Whether avian species can be infected with mammalian strains of Mycoplasma or vice versa has not been demonstrated, but little research has been done in this area.

Factors That Aggravate or Complicate M. Gallisepticum Infection

Certain Virus Diseases and Live-Virus Vaccination

Virus respiratory diseases are a part of air sac infection--the viruses of Newcastle disease (ND) and infectious bronchitis (IB) commonly occur in cases of this disease. Experimentally, ND and IB viruses have greatly increased the severity and rate of spread of M. gallisepticum infection.

Because live-virus vaccines for ND and IB have frequently been incriminated as stress factors in air sac disease, their use must be carefully considered. The decision to use them should be based on local conditions. Further, the eggs used to grow the ND and IB vaccine virus may harbor M. gallisepticum and this infection may be spread when the ND or IB vaccine virus is used. This possibility prompted a resolution from the Committee (see p. 8).

Escherichia coli and Other Pathogens

The respiratory tracts of normal birds have gram positive flora; those affected with air sac disease have high concentrations of gram negative flora that are mainly coliforms. As a complicating agent, one of the coliforms, E. coli, contributes most to clinical air sac infection. Pathogenic types of E. coli have been found in the intestinal tracts of normal birds, in litter, feed, and rodent feces, but the importance of these sources of bacteria in clinical air sac infection has not been determined. Bacteria causing coryza, fowl cholera, and other poultry diseases also complicate M. gallisepticum infection.

Environmental

Extreme or widely varying environmental conditions can predispose to or increase the severity of chronic respiratory disease (CRD). Low or fluctuating brooder temperatures, for example, have increased the severity and spread of M. gallisepticum infection and the severity of E. coli infection. Environmental stress can be minimized by good management.

DIAGNOSTIC TESTS FOR M. GALLISEPTICUM INFECTION

In chickens, signs of M. gallisepticum infection may be like those of Newcastle disease, infectious bronchitis, laryngotracheitis, fungus infection, and others. The usual signs are a nasal discharge, and a slight swelling below the eye. Coughing, sneezing, and a hoarse throat rattle may accompany these signs. Turkeys often have swollen sinuses with gelatinous exudate, watery eyes, and coughing with cheesy or cloudy air sacs. Since these signs are not specific, and since birds may be infected and therefore carriers without showing signs, specific tests for diagnosing the infection must be used. These specific tests usually require the services of a veterinary diagnostician. They are described here to show the weapons available in the fight against M. gallisepticum infection.

Serological Methods

It has been demonstrated unequivocally that chickens and turkeys infected with pathogenic M. gallisepticum develop blood components (antibodies) that will agglutinate a preparation containing the organism.

There are three agglutination tests that are useful in determining whether infection has taken place. These tests are the whole blood plate, serum plate, and tube agglutination. The hemagglutination inhibition test (H-I test), though highly reliable in the laboratory, is not practical for routine testing of flocks.

A flock diagnosis should not be made on the test results from a few birds. Serological tests should be used as supplements to other standards.

Bird Inoculation

Material harvested from respiratory cases of unknown etiology should be inoculated into the respiratory tract of three or more healthy birds immune to infectious bronchitis and Newcastle disease and free from M. gallisepticum infection. Within 7 to 21 days following inoculation, if the inoculum contained M. gallisepticum, one or more of the following signs may appear: Swollen sinuses, nasal discharge, tracheal rales, coughing, and slight loss of appetite. Gross pathology may include rhinitis, sinusitis, tracheitis, pneumonic lesions, and slight to extensive air sac inflammation. Serological tests 14 to 21 days after inoculation are essential to a correct diagnosis.

Embryo Inoculation

M. gallisepticum can be isolated and grown by yolk sac inoculation in embryonated chicken eggs (6 to 7 days of age). Eggs from flocks free of M. gallisepticum must be used. Primary passage in embryonated eggs followed by inoculation of artificial media is a common procedure.

Culture Methods in Artificial Media

Culture of M. gallisepticum in artificial media is generally more difficult than with routine bacterial infections. Special enriched media are essential and even these will not support growth of all strains. However, isolation of M. gallisepticum is possible in most attempts with the present procedures. For routine diagnostic use, the isolation on media may follow primary cultivation in embryonated eggs. Cultivation of the organism must be followed by its identification. Standards for the identification of M. gallisepticum isolated on artificial media are limited, but the following have been used:

The organism will grow only in a medium containing enrichments such as blood serum.

The growth in special fluid media is fine to granular and cloudiness, if present, is uniform after several days incubation at 37° C.

Broth smears contain small, round (coccoid) bodies when stained with Giemsa method, whereas other staining methods may fail to reveal

the organism. On solid culture medium (agar) the colonies are small and circular with a central dense papillae and can be differentiated by the Dienes staining method. Colonies not exceeding 0.5 mm. seem to grow into the agar and do not change on subculture.

Differentiation of *M. Gallisepticum* From Other Serotypes

This is accomplished by a summation of cultural and serological characteristics and pathogenicity. Many serotypes have been described but *M. gallisepticum* appears to be the prevailing pathogenic type in chickens and turkeys.

Histopathology

The microscopic picture produced by *M. gallisepticum* in chickens and turkeys provides satisfactory and valuable supporting evidence of infection. Lymphofollicular proliferation, although commonly found, is not pathognomonic (decisively characteristic) for the infection.

BASIC ESSENTIALS FOR CONTROL

Poultry disease investigators in colleges and universities and some commercial breeders have established flocks free of *M. gallisepticum* infection. Research unearthed the facts that made these achievements possible. The Committee's review of these facts, as presented in the previous sections, form the basis for its recommendations on how to control *M. gallisepticum*. The research facts further prompted the Committee to recommend management practices, as presented in the following sections, and these are also based on an understanding of the disease and its peculiarities. None of these recommendations can be safely ignored. Periodic testing of flocks that are being freed of *M. gallisepticum* infection and periodic testing of flocks that are already clean, and appropriate followups, for example, are necessary because:

1. *M. gallisepticum* infection is primarily transmitted through the egg. Dipping hatching eggs in suitable drugs or antibiotics reduces the incidence of egg transmission. The dipping, however, will not entirely eliminate egg transmission because no remedy, immunizing procedure, or other preventive or therapeutic practice in man, animal, or poultry has ever been 100-percent effective.

2. Similarly, certain drugs or antibiotic agents administered to adult breeders reduce the incidence of, but, again, will not entirely eliminate, egg transmission of *M. gallisepticum* infection.

3. Immunizing agents cannot be relied upon to eliminate egg transmission.

4. Serological tests satisfactorily detect infected flocks. But if a flock is tested and the tests are negative, the infection-free assumption is only valid for a short time. Periodic testing is therefore necessary to find out if the infection-free status is being maintained. When making test, at least 10 percent of the flock with a minimum of 100 birds should be tested. The Committee considers this recommendation mandatory.

Salvaging Special Blood Lines

The Committee's basic recommendations for establishing progeny free of M. gallisepticum infection from infected stock when desirable to salvage special blood lines are listed below. These recommendations should be integrated with the sanitation and management practices appearing on page 8.

1. Select birds with no clinical signs from groups with no active infection or from 2-year-old breeders.
2. Administer an effective drug or antibiotic to the selected birds by injection and follow with its use in the feed or water.
3. Dip hatching eggs in an effective drug or antibiotic.
4. Hatch eggs in small groups and raise poults or chickens in small isolated groups.
5. Examine cull chicks and pipped embryos for evidence of infection.
6. Examine cull turkeys or chickens during the growing period for lesions and serological evidence of infection.
7. Discard groups showing positive evidence of being infected.

Obtaining Replacement Breeders Free of M. Gallisepticum Infection

The Committee's basic recommendations for obtaining replacement breeders free of M. gallisepticum infection are listed below. These recommendations should also be integrated with the sanitation and management practices appearing on page 8.

1. Select replacement breeders from flocks clinically free of the infection. Hatch chickens from flocks that are clinically and serologically negative.
2. Use the random sample test or test the entire flock at the time of qualification for pullorum-typhoid testing. Discard flocks with positive serologic evidence of the infection.
3. Apply additional random sample tests after the regular test to enhance the success of the program. Test chickens at least twice at 3-to-4-months intervals. Test turkeys at least once within 2 months of the time they are obtained. In addition to the random sample test, cull chicks may be examined serologically.

Availability and Standardization of Antigens

The Committee emphasized that successful, widespread applications of serological testing programs depend on the availability of suitable antigens. Immediate steps must be taken to provide antigens to those States that do not now have a source. These antigens should meet certain standards as directed by a central agency.

The Committee felt that the Animal Disease Eradication Division of the Agricultural Research Service should undertake the manufacturing and standardization of diagnostic antigen for M. gallisepticum infection. A resolution reflecting this feeling was adopted.

Poultry Biologics

The Committee recommended that the Animal Inspection and Quarantine Division of ARS require that all eggs used in the production of poultry biologics (for example, the production of live-virus vaccines for Newcastle disease and infectious bronchitis) be obtained from flocks free of M. gallisepticum. Further, that all birds used to test these vaccines come from flocks free of M. gallisepticum infection and that these test birds must be serologically negative for M. gallisepticum at the end of the test period.

SANITATION AND MANAGEMENT PRACTICES TO CONTROL M. GALLISEPTICUM INFECTION

Breeder Flocks

1. Maintain breeder flocks on farms free of market flocks.
2. Avoid the introduction of eggs, poults, chicks, or adults from breeding stock until proved free of M. gallisepticum infection.
3. Prevent transmission from outside sources by indirect contact through contaminated equipment, footwear, clothing, vehicles, or other mechanical sources.
4. Provide adequate isolation of breeder flocks to avoid air-borne transmission from infected flocks.
5. Minimize contact of breeder flocks with game and free-flying birds.
6. Eliminate other fowl from breeder farm.
7. Keep the rodent population and other pests under control.
8. Tailor vaccination programs to needs of farm and area.

9. Allow no visitors except under controlled conditions.
10. Clean and disinfect equipment after each use.
11. Provide clean footwear and provide an adequate security program.
12. Clean and disinfect houses between flocks.
13. Use well-drained range.
14. Use clean, dry litter free of mold.
15. Keep accurate records of death losses.
16. Dispose of all dead birds by burning, deep burial, or by putting them in special disposal pits.
17. Seek services of veterinary diagnostician if abnormal losses or signs of disease occur.
18. Adopt and maintain a clean egg program.
19. When adding new buildings or remodeling old ones, consider the special recommendations of the Committee on page 10.

Market Flocks

1. Clean and disinfect brooding equipment and buildings between each group. The reuse of litter should not be considered when initiating a broiler-growing program of flocks free of M. gallisepticum infection.
2. Obtain day-old poults or chicks from breeder flocks free of M. gallisepticum infection.
3. Separate age groups as completely as possible during brooding and growing periods.
4. Prevent contact with other fowl.
5. Prevent introduction of infection from outside sources through contaminated equipment, footwear, clothing, vehicles, and other sources.
6. Allow no visitors.
7. Provide clean footwear and an adequate security program.
8. Keep rodent population and other pests under control.
9. Dispose of all dead birds properly.
10. Keep accurate mortality and medication records.
11. Provide for a break in production each year of at least 2 to 4 weeks for a complete cleanup of equipment, premises, and buildings.

12. Tailor vaccination programs to needs of farm and area.
13. Seek services of veterinary diagnostician if abnormal losses or signs of disease occur.

RECOMMENDATIONS RELATIVE TO BUILDINGS AND EQUIPMENT

Buildings and equipment should be designed to facilitate sanitation and to insure some degree of environmental control. The following are recommended:

1. Easily cleanable floors such as concrete.
2. Adequate floor drains with effective disposal systems.
3. Adequate insulation with a vapor barrier on the interior. The barrier should be protected from physical damage by the birds, equipment, or cleaning and management activity.
4. Adequate ventilation, sized to control moisture, dust, ammonia, carbon dioxide, and other air contaminants.
5. Temperature controlled to prevent excessive heat or cold stresses on poultry.
6. Feed bins, central heat equipment, electric and other meters should be serviceable from outside the poultry house.
7. Security locks provided at all entry points.
8. Screens and shields to keep out rodents and wild birds.
9. Washable interior that is smooth, durable, and relatively impervious to water.
10. Adequate water supply for wash downs.
11. Adequate and foolproof feeding, watering, and medication facilities.
12. The site should be well arranged for drainage, litter removal, and should be kept clean of rubbish and dead birds. Orientation, accessibility for service, and isolation from other poultry are important considerations.
13. Lighting should be adequate to permit inspection of birds and facilities.
14. Equipment controls should be simple and effective.
15. Facilities should be provided for cleaning and disinfecting equipment.
16. Clean source of drinking water.

MORE RESEARCH NEEDED

The Committee recommended that research into all the ramifications of M. gallisepticum infection should be extended and intensified. Specific recommendations follow:

To Improve Isolation and Identification

1. Continue basic studies to determine the nutrient requirements and the metabolism of avian Mycoplasma, including both pathogenic and nonpathogenic groups. Include in these studies freshly isolated strains as well as those adapted to laboratory propagation. A team approach among several disciplines is recommended.

2. Develop criteria for classification and standardization. Utilize electron microscopy for detailed morphological characterization of avian Mycoplasma.

3. Investigate tissue culture systems further for the isolation and propagation of Mycoplasma.

4. Investigate the use of fluorescent antibody to determine distribution of the organism within hosts.

5. Further explore pathogenic and nonpathogenic strains and their behavior in embryonated eggs.

6. Develop improved antigens.

7. Investigate the factors influencing specificity and sensitivity of tests. Include antigen factors as well as specific and nonspecific host responses.

8. Study histopathological changes produced by all strains of avian Mycoplasma.

9. Correlate positive serological reactions with isolation of the organism.

To Determine Host Range and Other Characteristics

1. Determine the host range of known avian serotypes and the susceptibility of avian species to each serotype.

2. Identify the role of free-flying birds in the dissemination of pathogenic avian serotypes.

3. Establish the carrier status of mammals harboring avian serotypes.

4. Determine survival potential of avian Mycoplasma under various environmental conditions.

5. Determine survival potential in body fluids, excretions, and poultry carcasses.

6. Determine response of the agent to the commonly used disinfectants and sanitizing procedures.

To Improve Immunizing Methods

1. Carry out further work in chickens and turkeys with emphasis on improvement of immunizing methods for M. gallisepticum infection.

2. Determine the relationship between immunity and circulating antibodies.

To Define Further the Factors That Aggravate or Complicate the Infection

1. Investigate more thoroughly the role of viruses in air sac disease.

2. Study means of minimizing the complicating effect of live-virus vaccines.

3. Determine means of transmissions of coliforms.

4. Determine the long-term effect of coliform infections.

5. Determine the role of other pathogens in air sac disease and airsacculitis.

6. Determine the optimum environments for poultry and the specific effect of deviations therefrom on air sac disease and airsacculitis.

To Understand More About Transmission

1. Explore the mechanism of egg transmission more extensively.

2. Explore the susceptibility of the reproductive tracts of immature and mature birds to Mycoplasma infection.

3. Using naturally and artificially infected birds, study the localization of the agent in tissue and its persistence in and elimination from the body.

4. Explore more extensively the possibility of incubator transmission of M. gallisepticum infection.

5. Investigate air-borne transmission under natural and artificial exposures.

SPECIAL NOTE

Many of the Committee members expressed the belief that continued progress against M. gallisepticum infection depends on more than expanded and intensified research and the dissemination of research findings. Research workers, extension workers, and regulatory officials within an area or State must maintain close liaison so the incidence of the disease can be known at all times and mutual problems can be handled together. Similarly, flock owners are asked to cooperate with research workers and regulatory officials so the control of M. gallisepticum infection will become a reality instead of an unresolved problem.

GLOSSARY

Mycoplasma is the scientific name for a group of micro-organisms, smaller than the common bacteria but larger than viruses, that occur in man, livestock, and poultry.

Mycoplasma gallisepticum is the scientific name for the specific type of Mycoplasma that causes Chronic Respiratory Disease in chickens and Infectious Sinusitis of turkeys.

Air Sac Disease is the disease complex that occurs when Mycoplasma gallisepticum infection is complicated by secondary infections. This disease complex should more properly be designated complicated CRD.

CRD (Chronic Respiratory Disease) is the name given to the respiratory disease produced by Mycoplasma gallisepticum in chickens.

Infectious Sinusitis is the name given to the respiratory disease produced by Mycoplasma gallisepticum in turkeys.

Airsacculitis is any inflammatory reaction of the air sacs. This may be due to a wide variety of causes, including Mycoplasma gallisepticum.

PPLO (Pleuropneumonia-like organism) is the name formerly used for the group of organisms now known as Mycoplasma.

COMMITTEE MEMBERS

Ahrens, M. C.	USDA, Agricultural Engineering Research Division, Beltsville, Maryland
Barber, C. W.	North Carolina State College Raleigh, North Carolina
Benton, W. J.	University of Delaware Newark, Delaware
Bierbower, G. S.	USDA, Agricultural Marketing Service, Beltsville, Maryland
Blake, J. T.	Utah State College Logan, Utah
Boyer, C. J., Jr.	Cornell University Ithaca, New York
Chute, H. L.	University of Maine Orono, Maine
Cover, M. S.	University of Delaware Newark, Delaware
Craig, F. R.	North Carolina State College Raleigh, North Carolina
DeCamp, Daniel	HEW, Food and Drug Administration Washington, D. C.
DeVolt, H. M.	University of Maryland College Park, Maryland
Dickinson, E. M.	Oregon State University Corvallis, Oregon
Drury, L. N.	USDA, Agricultural Engineering Research Division, Athens, Georgia
DuBose, R. T.	Virginia Polytechnic Institute Blacksburg, Virginia
Dunlop, W. R.	University of New Hampshire Durham, New Hampshire
Edds, G. T.	University of Florida Gainesville, Florida
Fabricant, Julius	Cornell University Ithaca, New York
Frey, M. L.	University of Wisconsin Madison, Wisconsin

Gale, Charles	Ohio Agricultural Experiment Station Wooster, Ohio
Geleta, J. N.	HEW, Food and Drug Administration Washington, D. C.
Gentry, R. F.	Pennsylvania State University University Park, Pennsylvania
Gill, Eugene	HEW, Food and Drug Administration Washington, D. C.
Griffin, J. G.	USDA, Agricultural Engineering Research Division, State College, Mississippi
Gross, W. B.	Virginia Polytechnic Institute Blacksburg, Virginia
Grumbles, L. C.	Texas A & M College College Station, Texas
Hall, C. F.	Texas A & M College College Station, Texas
Hamilton, C. M.	Western Washington Experiment Station Puyallup, Washington
Hanson, L. E.	University of Illinois Urbana, Illinois
Heddleston, K. L.	USDA, Animal Disease and Parasite Research Division, NADL, Ames, Iowa
Hendrix, A. T.	USDA, Agricultural Engineering Research Division, Athens, Georgia
Hopkins, S. R.	USDA, Animal Disease and Parasite Research Division, SPRL, Athens, Georgia
Jenkins, Wood	USDA, Federal Extension Service Washington, D. C.
Junnla, W. A.	USDA, Agricultural Engineering Research Division, St. Paul, Minnesota
Kleckner, A. L.	University of Georgia Athens, Georgia
Krauss, W. C.	University of Delaware Newark, Delaware
Luginbuhl, R. E.	University of Connecticut Storrs, Connecticut
McCune, E. L.	University of Missouri Columbia, Missouri
McKee, G. S.	USDA, Agricultural Marketing Service Washington, D. C.

McLaughlin, D. K.	USDA, Animal Disease and Parasite Research Division, BPL, Beltsville, Maryland
Moore, Sam A.	USDA, Animal Husbandry Research Division Beltsville, Maryland
Mora, E. C.	Auburn University Auburn, Alabama
Morris, Dank	Georgia Poultry Laboratories Oakwood, Georgia
Moses, H. E.	Purdue University Lafayette, Indiana
Olesiuk, Olga	University of Massachusetts Amherst, Massachusetts
Olson, N. O.	West Virginia University Morgantown, West Virginia
Omohundro, R. E.	USDA, Animal Disease Eradication Division Washington, D. C.
Oshel, D. D.	USDA, Animal Inspection and Quarantine Division, NADL, Ames, Iowa
Osteen, O. L.	USDA, Animal Disease and Parasite Research Division, Beltsville, Maryland
Ota, H.	USDA, Agricultural Engineering Research Division, Beltsville, Maryland
Parkhurst, R. T.	USDA, Animal Husbandry Research Division, SCPRL, State College, Mississippi
Patterson, W. C.	USDA, Animal Disease and Parasite Research Division, SPRL, Athens, Georgia
Peacock, G. V.	USDA, Animal Inspection and Quarantine Division, Washington, D. C.
Peterson, E. H.	University of Arkansas Fayetteville, Arkansas
Pomeroy, B. S.	University of Minnesota St. Paul, Minnesota
Rollins, W. L.	Colorado State University Fort Collins, Colorado
Schmittle, S. C.	Georgia Poultry Disease Research Laboratory University of Georgia Athens, Georgia
Schoenhard, D. E.	Michigan State University East Lansing, Michigan

Shaklee, W. E.	USDA, Cooperative State Experiment Station Service, Washington, D. C.
Stephens, James F.	Clemson College Clemson, South Carolina
Teter, N. C.	USDA, Agricultural Engineering Research Division, Beltsville, Maryland
Thompson, C. H., Jr.	USDA, Cooperative State Experiment Station Service, Washington, D. C.
Van Roekel, H.	University of Massachusetts Amherst, Massachusetts
Vardaman, T. H.	USDA, Animal Disease and Parasite Research Division, SPRL, Athens, Georgia
Walker, John W.	USDA, Animal Disease Eradication Division Washington, D. C.
Worcester, W. W.	California Department of Agriculture Sacramento, California
Yamamoto, R.	University of California Davis, California
Yoder, H. W., Jr.	Iowa State University Ames, Iowa
Zumbro, P. B.	USDA, Animal Husbandry Research Division Beltsville, Maryland



Growth Through Agricultural Progress